Abstract

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Title of diploma thesis: Development of HPLC method for determination of vitamin B_1 in clinical research

The present thesis describes the development and partial validation of the new HPLC method for determination of vitamin B1 (thiamine hydrochloride) and its phosphorylated derivatives (thiamine monophosphate, thiamine pyrophosphate). This method is based on the use of liquid chromatography with indirect fluorescence detection. Separation was achieved using modern second generation monolithic stationary phase High Resolution Chromolith RP-18e 100 x 4.6 mm (Merck, Germany) in combination with gradient elution. Mobile phase was consisting of the mixture of 95% phosphate buffer 10 mmol / l pH 6 5 and 5% acetonitrile (ACN), flow rate of the mobile phase was 2.5 ml / min, from 2.1. min 4 ml / min. For fluorescence detection of thiamin hydrochloride (TH), thiamin monophosphate (TMP) and thiamine pyrophosphate (TPP, thiamine diphosphate) was necessary to use chemical oxidation by potassium ferricyanide to thiochrome and its adequate esters having photoluminescence activity. Chromatographic method has been partially validated. Peak symmetry, Column efficiency, Peak resolution, Limit of detection and quantification, Repeatability of extraction and derivatisation were determined. Project will continue by focusing on the urine and serum samples pretreatment procedure, to effort the simple and short preanalytical phase, which will be used for patients of 3rd Internal Gerontometabolic Clinic, University Hospital in Hradec Králové.